Energy Conservation in a Biogeochemically Significant Microbial Mutualism

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ESPP2 Environmental Stress Pathway Project

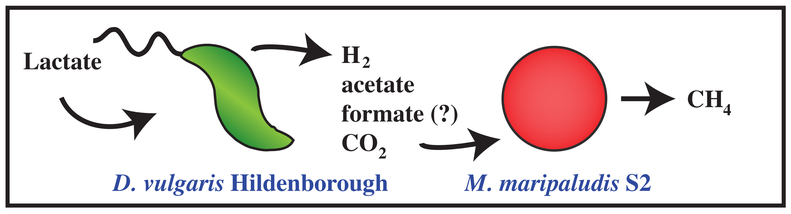
DOE GENOMICS:GTL SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT
OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival — http://vimss.lbl.gov/

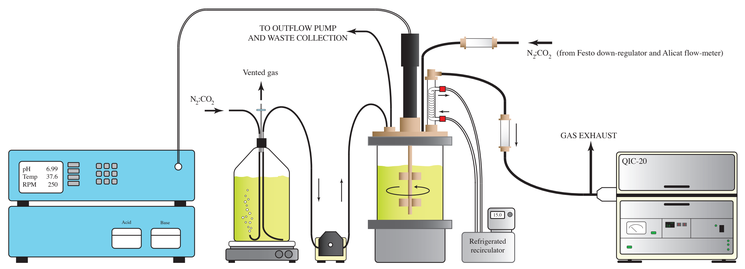
ABSTRACT

Complex interactions govern microbial communities in both pristine and contaminated environments. Unfortunately, a limited understanding exists regarding the interactions sustaining these communities. Without a deeper knowledge of the molecular basis driving the community structure and function, bioremediation of contaminated sites suffers from inefficient or ineffective design strategies. The VIMSS/ESPP2 project aims at resolving the molecular basis for microbial organisms and communities found at heavy-metal contaminated environments, such as the Hanford 100H site. Sulfate-reducing microbes (SRM) commonly compose significant fractions of the microbial community in contaminated anaerobic sites. The VIMSS/ESPP2 project extensively examined a representative SRM, *Desulfovibrio vulgaris* Hildenborough, building a detailed understanding of stress response mechanisms through intensive monoculture study. However, *D. vulgaris* often populates environments deficient in sulfate, relying upon syntrophic associations with hydrogenotrophic methanogens for continued growth. Investigation of an archetypical community composed of *D. vulgaris* Hildenborough and a representative methanogen, *Methanococcus maripaludis* S2, serves as a basis for understanding the physiological differences between growth modalities. Using transcriptional analysis, we demonstrate that continuously grown cultures of *D. vulgaris* Hildenborough, up-regulate a broad suite of electron transfer enzymes during syntrophic growth. Mutational analyses indicate keys roles for four enzymes (Coo, Hmc, Hyd and Hyn) not essential for sulfate-respiration. Specifically, these results provide a developed molecular basis for understanding this "community of two" while also serving as the foundation for future VIMSS/ESPP2 community analysis. More generally, these results suggest syntrophic growth and sulfate-respiration rely upon largely independent energy generation pathways.

SYNTROPHIC ASSOCIATION

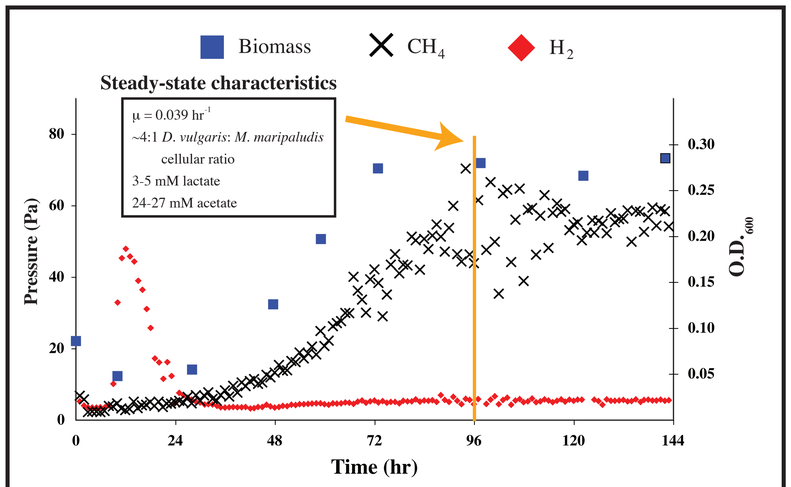


CHEMOSTAT CONFIGURATION



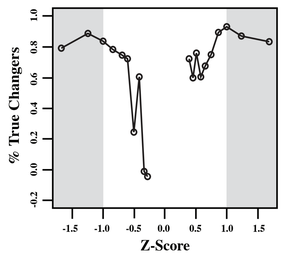
Chemostats are run at 37 ºC with a stirring speed of 250 rpm. The headspace of the chemostat is flushed with a mixture of N_2:CO_2 (90:10) at a rate of 0.20 ml/min. The headspace gas composition is sampled in 15 min. intervals using a Hiden QIC-20 mass spectrometer.

BIOMASS PRODUCTION

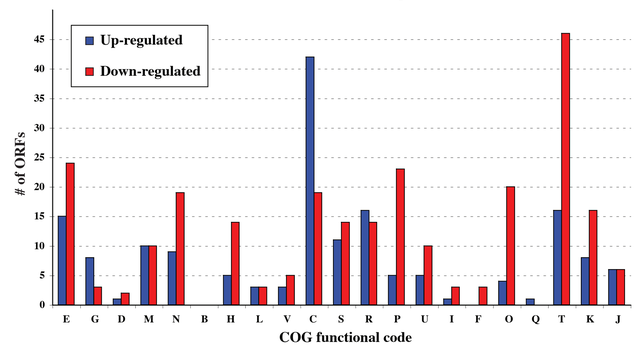


Representative biomass, hydrogen and methane profiles for a coculture biological replicate. Continuous culture began at hour 96 (orange bar). Biomass from triplicate biological replicates of both the coculture and sulfate-limited monocultures was analyzed by the ESPP2 Functional Genomics Core using whole-genome microarrays containing duplicate spots of each open-reading frame (ORF). At least three slides were used for each biological replicate. The ESPP2 Computational Core calculated RNA/DNA expression ratios for each ORF and determined $\log_2$ ratios (coculture/monoculture). Z-scores for each ORF were used for assessing statistical significance. Operon-based estimates of local accuracy indicated an absolute Z-score of 1.0 accurately predicts expression changes. Using this value, statistically significant up-regulation of 169 ORFs and down-regulation of 254 ORFs occurred.

TRANSCRIPTIONAL ANALYSIS



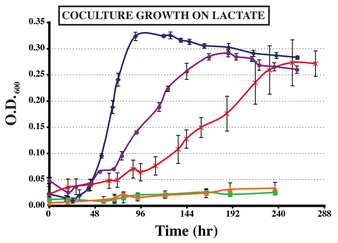
Operon-based estimtes of local accuracy (left figure) and clusters of orthologous groups (COGs, bottom figure) up- and down-regulated during syntrophic growth. Categories are amino acid transport (E), carbohydrate transport and metabolism (G), cell division and chromosome partitioning (D), cell envelope biogenesis (M), cell motility and secretion (N), chromatin structure and dynamics (B),coenzyme metabolism (H), DNA replication, recombination and repair (L), defense mechanisms (V), energy production and conservation (C), function unknown (S), general function prediction only (R), inorganic ion transport and metabolism (P), intracellular trafficking and secretion (U), lipid metabolism (I), nucleotide transport and metabolism (F), post-translational modification, protein turnover, chaperones(O), secondary metabolites biosynthesis, transport and catabolism (Q), signal transduction mechanisms (T), transcription (K) and translation, ribosomal structures and biogenesis (J)

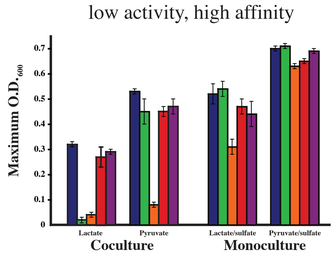


MUTATIONAL ANALYSIS

D. vulgaris Hildenborough mutants were screened for phenotypic growth effects in both cocultrure and monoculture. Cocultures were grown on lactate and pyruvate. Monocultures grown on lactate/sulfate and pyruvate/sulfate. Error bars represent standard deviation of three replicates.

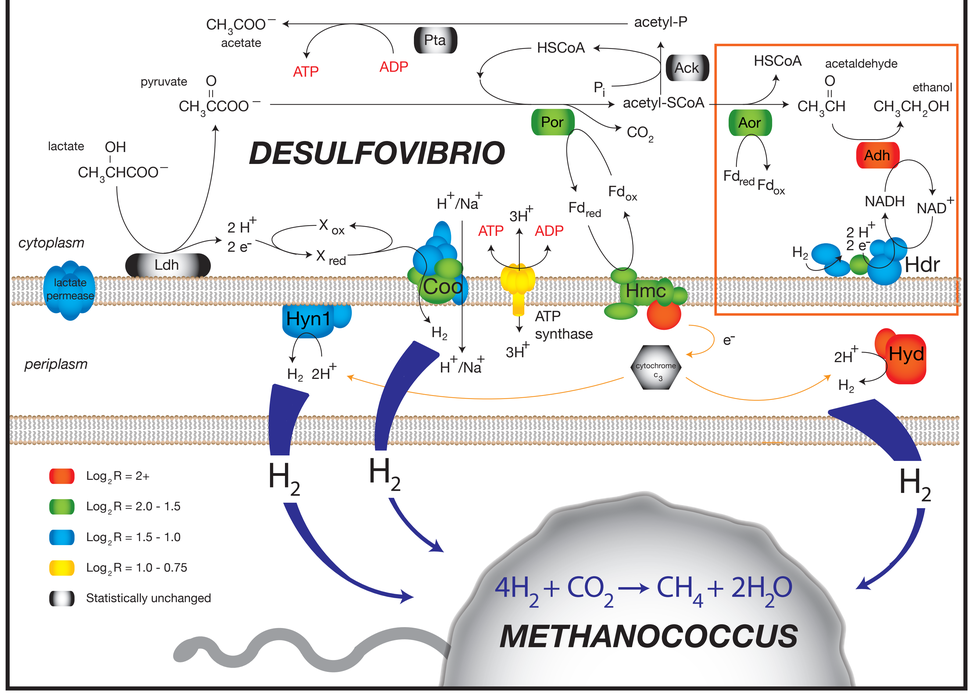
STRAIN	MUTANT	SYMBOL	ORFs	DESCRIPTION
wildtype	———	◆	———	———
JW3034	Δ*cooL*	■	DVU2288	putative periplasmic hydrogenase, [NiFe] small subunit
H801	Δ*hmc*	▲	DVU0531-6	high-molecular weight cytochrome complex
Hyd100	Δ*hydAB*	×	DVU1769-70	[Fe] containing periplasmic hydrogenase high activity, low affinity
NiFe100	Δ*hynAB-1*	●	DVU1921-2	[NiFe] containing periplasmic hydrogenase low activity, high affinity





PROPOSED SYNTROPHIC GROWTH MODEL

Proposed metabolic model for syntrophic growth for *D. vulgaris* Hildenborough. Color scheme refers to transcriptional changes of individual genes during co-culture growth versus sulfate-limited monoculture. X represents an unknown carrier interacting with Ldh. Abbreviations: Ldh – lactate dehydrogenase (DVU0600), Por – pyruvate:ferredoxin oxidoreductase (DVU3025), Ack – acetate kinase (DVU3030), Pta – phosphate acetyltransferase (DVU3029), Aor – aldehyde:ferredoxin oxidoreductase (DVU1179), Adh – alcohol dehydrogenase (DVU2405), Hdr – heterodisulfide reductase (DVU2399 – 2404), Fd – reduced or oxidized ferredoxin, Coo – CO-induced hydrogenase (DVU2286-93), Hmc – high molecular weight cytochrome complex (DVU0531-6), Hyn1 – [NiFe] hydrogenase isozyme 1 (DVU1921-2), Hyd – [Fe] hydrogenase (DVU1769-70). Orange box depicts hypothetical pathway of ethanol production (via hydrogen consumption).



CONCLUSIONS, IMPLICATIONS, FUTURE WORK

- **Syntrophic growth and sulfate-respiration rely upon independent electron transfer systems**
  - possible explanation of H_2 burst observed in monoculture
- Functional related enzymes present in sequenced genomes of syntrophs
  - Several obligate syntrophs contain remnants of sulfate-respiration pathway

  Did syntrophy evolve from ancient SRM populating environments stably devoid of terminal electron acceptors?
- Builds base community for analyzing more complex interactions
  - add acetoclastic methanogens, Fe-reducers, cellulolytic, etc.
- Determine if community shift observed at Hanford 100H site displays similar transcriptional features

ACKNOWLEDGMENT

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